Cribra Orbitalia and Trace Element Content in Human Teeth From Neolithic and Early Bronze Age Graves in Southern Poland

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ABSTRACT Determination of element levels in bones and teeth can complement knowledge of the diagnostics and etiology of various diseases in prehistoric populations. Calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), and lead (Pb) content were analyzed in teeth from human skeletons dated to 3,000–1,400 BC from Malopolska Upland loess. Levels of iron and calcium were determined using atomic absorption spectroscopy (AAS), and lead, cadmium, and copper levels were measured using anodic stripping voltametry (ASV). Molar teeth from specimens with cribra orbitalia were selected for analyses, and teeth from specimens with no pathological changes were used as a control. No significant correlations between the content of particular elements and the tooth class, specimen age, or depth of burial pit were observed. The Fe content in specimens with cribra orbitalia is not the best measure for this disease's etiology. Thus, interelement correlations and proportions might give a better picture of the biological condition of the specimen and of the investigated groups. Am J Phys Anthropol 103:201–207, 1997.

Modern paleoanthropological research increasingly uses chemical and physical methods to analyze bone material and to broaden and complement information and knowledge on the biological condition of human groups, prehistoric diets, and the etiology of various diseases (Ruff, 1992; Sandford, 1992; Wolsperger, 1992; Klepinger, 1992; Klepinger, 1993; Ambrose, 1993; Stuart-Macadam, 1989).

Anthropological applications of element analyses are important in investigating the relationships between nutrition and disease (Walker, 1986) and estimating the health effects of trace element deficiencies or excesses in human tissues. Diet, cultural customs, and environmental levels of trace elements in soil and water may influence element uptake (Sandford, 1992). Element analysis has been used to investigate toxic pollutants—for example, lead (Pb) exposure in historical populations (Waldron et al., 1976; Whittaker and Stack, 1984)—or to explore the source of specific nutritional deficiencies among ancient peoples (Sandford, 1993; Sandford and Kissiling, 1993).

A very interesting problem for prehistoric research is the occurrence of cribra orbitalia. Investigations of the etiology of cribra orbitalia have been based on estimating bone iron concentrations in different populations (Zaino, 1968; Grupe, 1995; Stuart-Macadam, 1985). Waldron et al. (1976) also

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Fig. 1. Region of loess upland, Malopolska Upland. Hatched area, limits of region.

confirmed the influence of skeletal lead levels on pathological changes.

Bone changes such as cribra orbitalia and porotic hyperostosis occurring in adults are probably the result of bone marrow hypertrophy in childhood. Individuals with severe anemia may not live beyond childhood (Stuart-Macadam, 1985). Development of cribra orbitalia and the formation and growth of the first and second permanent molars take place at the same time. Thus, teeth can record the "history" of childhood.

The aim of this study is to investigate iron (Fe), lead (Pb), copper (Cu), cadmium (Cd), and calcium (Ca) levels in Neolithic and Early Bronze Age skeletons from loess uplands in the south of Poland (Malopolska Upland) dated 3,000–1,400 BC (Gleń-Haduch, 1995) and to test hypotheses concerning the association between element concentrations and pathology.

ARCHEOLOGICAL CONTEXT

The Malopolska Upland region of Poland (Fig. 1) has always been attractive for settlement because of its ideal natural conditions. During the Neolithic and the Early Bronze Age period the climate was subboreal, and steppe and forest-steppe flora developed on fertile soils (Kruk, 1980).

Gathering, hunting, agriculture, and animal husbandry were the basic sources of sustenance. The diet of the human populations inhabiting the Malopolska Upland was varied and probably contained all the components necessary for proper physiological functioning.

Burial pits of the Funnel-Beaker culture were found at the Neolithic site in Bronocice (Kielce Voivodeship) and are dated to 3,000–2,400 BC (radiocarbon dating [Kruk and Milisauskas, 1983]). Most of the burial pits contained ceramics, stone and animal bone tools, and shell adornments. No metal objects were found (Tarnobrzeg Voivedeship) dated to 1,900–1,800 BC included ceramics and stone tools only (unpublished data).

In Szarbia human remains were found in Mierzanowicka culture graves and settlement pits from the Early Bronze Age. Radiocarbon dating locates the Szarbia site between 1,700 and 1,400 BC (Baczyńska, 1993). The graves contained clay vessels, stone tools and shell, faience, and in some cases bronze adornments.

In contrast to the local origins of the populations representing the Funnel-Beaker culture (Bronocice) and the Mierzanowicka culture (Szarbia), the group representing the Bell-Beaker culture (Samborzec) did not originate in central Europe. This deduction is shown by both anthropological (Gleń-Haduch, 1990) and archeological data (Machnik, 1979). The Bell-Beaker culture, of pan-European range, probably arose on the Iberian peninsula, but it also shows links with southwestern Asia (Machnik, 1979). The Bell-Beaker populations were highly mobile, covering considerable distances within short periods of time, and were engaged in hunting, animal husbandry, and trade.

The skeletons employed in our study were excavated at different depths (20–200 cm) beneath the contemporary soil level. The burial pits were filled with accumulated loess and soil. At all the investigated archaeological sites, the physical and chemical soil parameters were identical. The loess in which the remnants of all the investigated specimens were buried had a pH of 6.8–7.6 and also had a very high sorption capacity in

its surface layers. It therefore contained a high amount of stable compounds, and the element mobility was low (Kabata-Pendias and Pendias, 1979). The neutral or slightly alkaline character of the soil, the very good preservation of the teeth, and the high percentage of elements in the surrounding soil suggest that diagenetic processes had very little effect on the material excavated for investigation.

MATERIAL AND METHODS

Our focus was on skulls with cribra orbitalia and porotic hyperostosis. To define the severity of porotic hyperostosis, we used Hengen's (1971) seven-increment scale. All skulls with signs of cribra orbitalia that were used for subsequent analyses had second-degree changes—a number of tiny holes and deeper grooves in the tabula externa of the orbital roof. The holes were up to 1 mm in diameter, which corresponds to code 6.2.1 in Buikstra and Ubelaker (1994). We also examined skeletons without pathological changes recovered from the same sites. The latter remains were used as a control group in our analyses.

Thirty-one teeth were analyzed: 23 M1, 6 M2, 2 m2. Eleven teeth were obtained from females (25–60 years), 11 from males (20–60 years) and nine from subadults (3–18 years). Seventeen teeth were obtained from individuals with cribra orbitalia and 14 from those without pathological changes. All teeth used for the analyses were very well preserved and were without signs of caries. No visible enamel or cement cracks or enamel hypoplasia were observed.

Laboratory methods

After extraction, the teeth were vigorously cleaned and brushed with deionized water to remove superficial organic and inorganic materials in an ultrasonic cleaner. Deionized water of 18 Mhom/cm resistivity was prepared using a Millipore water purification system. After cleaning, the samples were dried in an incubator at 80°C for 60 min. Each tooth was thoroughly crushed in an agate mortar to obtain small fragments. The samples were digested in a 4:1 mixture of concentrated nitric acid (65% Suprapur; Merck, Darmstadt, Germany) and perchloric acid (70% Suprapur; Merck) on a hotplate for 48 h until all samples were completely dissolved. The samples were then placed in 10 ml of spectrally pure water (no trace elements had been detected in this water). Blanks were prepared by the same method.

The concentration of Ca and Fe were estimated in a graphite furnace atomic absorption spectrometer (AAS 1; Carl Zeiss, Jena, Germany). Data were calculated using a calibration curve prepared with nitric acid and perchloric acid solutions using the same concentrations as for the sample. The values were averaged from three single AAS sample injections. The standard deviations were 3–5% for each element. The lower detection limit for Fe was 0.1 µg/ml and for Ca 0.25 µg/ml.

Lead and copper were determined by anodic stripping voltammetry (ASV) with a mercury-plated was-impregnated graphite electrode (Karai et al., 1980; Wang, 1985). The lower detection limit for Pb and Cu was below 0.0001 µg/ml.

The reference analysis for blanks was of a value lower than the detection limit of the method. The concentrations were expressed as micrograms per gram of dried sample.

Statistical analyses

The data obtained through the chemical investigation (Table 1) were subjected to statistical analyses. These indicated that the concentrations of all analyzed elements except for Ca, both at individual sites and generally, are not normally distributed. For this reason the lead, copper, and iron concentrations were logarithmically transformed into normal distributions. Further statistical analysis involved a multifactor analysis of variance method using the Bonferroni test as the range test. The analysis examined three factors (cribra orbitalia/normal, archeological site, and sex) and the concentrations of Pb, Fe, Cu, and Ca. A possible relationship between the depth of graves and the concentration of elements was investigated with regression analysis. The same approach was used to determine any association between the age of individuals and the level of the

TABLE 1. Concentration of elements in human teeth from individuals with pathological and without pathological changes from the Neolithic and Early Bronze Age cemeteries from South Poland

Site	Fe (μg/g)		Pb (μg/g)		Cu (μg/g)		Ca %	
	Cribra	Norma	Cribra	Norma	Cribra	Norma	Cribra	Norma
Bronocice (Funnel Beaker), 3000–2200 BC								
N	8	5	8	5	8	5	8	5
Mean	21.38	15.95	0.61	0.32	2.91	1.43	26.56	24.94
SD	9.39	1.47	0.25	0.07	1.41	0.56	4.34	0.53
Samborzec (Bell Beaker), 1900–1800 BC								
N	5	4	5	4	5	4	5	4
Mean	21.76	18.38	0.32	0.23	1.59	2.21	27.66	26.92
SD	13.93	5.20	0.05	0.02	0.26	0.91	3.07	3.39
Szarbia (Early Bronze), 1700–1400 BC								
N	4	5	4	5	1	5	4	5
Mean	15.09	15.75	0.75	0.25	0.83	0.99	22.40	25.76
SD	6.5	5.40	0.33	0.04	—	0.20	2.96	2.87
Total								
N	17	14	17	14	14	14	17	14
Mean	20.01	16.57	0.56	0.27	2.29	1.49	25.90	25.80
SD	10.14	4.16	0.27	0.06	1.29	0.74	4.06	2.44

elements in their hard tissues. Finally, linear regression was used to test the relationship between Pb, Cu, and Fe for individuals with cribra orbitalia and individuals without visible pathological changes.

RESULTS AND DISCUSSION

Distribution of pathology

Among the Neolithic remains, 27.6% of the specimens displayed cribra orbitalia, and, among the Early Bronze Age skulls from the Malopolska Upland, 20.8% of had cribra orbitalia (Gleń-Haduch, 1995). There was no difference between juveniles and adults in the frequency of occurrence of cribra orbitalia in the Neolithic group. However, the pathology was more frequently observed in Neolithic male skulls than in the female skulls. In the Early Bronze Age group there were no children with cribra orbitalia.

Element concentration by site, depth of burial, sex, and pathological status

The results of the chemical analysis of teeth from the investigated sites are listed in Table 1. The findings from the multifactor analysis of variance are shown in Table 2.

Concentration by site, depth of burial, and age of specimens. We found no difference between the levels of a particular element between sites in any of the investigated cases (Table 2).

TABLE 2. Multifactor analysis of variance analysis results, with Bonferroni correction

	Pb (μg/g)		Fe (μg/g)		Cu (μg/g)		Ca %	
	F	*P*	F	*P*	F	*P*	F	*P*
Cribra/norma	28.26	0.0001*	0.92	0.35	0.93	0.35	0.63	0.44
Site	2.72	0.10	0.39	0.68	2.45	0.12	0.97	0.39
Sex	0.45	0.64	3.36	0.04*	0.35	0.70	0.99	0.38

*Statistical significant differences $P < 0.05$.

No differences in iron content were found between the investigated archaeological sites. The fact that all the skeletons were preserved in very similar soil conditions, at the same pH, and that teeth preservation was the same in specimens with pathological changes related to cribra orbitalia, may suggest that diagenetic processes had little influence on iron content variability. This was first confirmed by Zaino (1968), who showed that for many regions, such as loess uplands where ancient bones are well preserved, the level of bone iron is the same at the time of excavation and the time of death, exchangeable iron concentrations are low, and diagenetic remodelling has not influenced iron exchange.

Lead and copper levels in the teeth of the investigated human groups were generally low. Lead and copper content in the loesses

TABLE 3. Correlations between depths of graves (X) and concentrations of elements

Site	Fe (μg/g)	Pb (μg/g)	Cu (μg/g)	Ca %
Bronocice (Funnel Beaker), 3000–2200 BC				
N	13	13	13	13
R^2	0.013	0.20	0.16	0.22
F	0.10	2.11	1.60	2.32
Samborzec (Bell Beaker), 1900–1800 BC				
N	9	9	9	9
R^2	0.12	0.05	0.18	0.07
F	1.02	0.41	1.54	0.59
Szarbia, 1700–1400 BC				
N	9	9	6	9
R^2	0.14	0.002	0.04	0.012
F	1.20	0.01	0.18	0.09

TABLE 4. Correlations between age of individuals (X) and level of elements (Y)

Element	N	R^2	F
Fe (μg/g)	31	0.0152	0.51
Pb (μg/g)	31	0.001	0.03
Cu (μg/g)	28	0.0125	0.41
Ca %	31	0.0085	0.28

of southern Poland and thus the soils in which the skeletons were found is very stable and low (Kabata-Pendias and Pendias, 1979). The soil also contains a small amount of organic matter. These conditions strongly influence changes in the quantities of elements in postmortem bone material.

On the other hand, a significantly higher level of Cu was found in three skeletons from the site in Szarbia. The skeletons were recovered from graves that were richly furnished with bronze objects, which probably caused a saturation of the bones and teeth with copper compounds. The teeth of such specimens were excluded from further analyses.

Cadmium content in all the analyzed samples was very low, below the method's sensitivity limits. This fact can be explained by the very low exposure of the investigated human populations to this toxic elements.

No relationship could be demonstrated between the depth of the burial pit and the level of a particular element (Table 3). No relationships were found between the content of Pb, Cu, Ca, and Fe and the age of the specimens (Table 4).

Element concentrations by tooth type, sex, and pathology. There were no statistically significant differences in the content of particular elements by the type of tooth studied (results not shown). Similar observations have been made in contemporary human populations (Bercovitz and Laufer, 1990).

Although iron levels do not differ significantly between individuals with cribra orbitalia and without (Table 2), we observed higher variability in Fe levels in specimens with pathological changes (Table 1). The higher variability coefficients and standard deviations could be the result of disturbed homeostasis. Certainly, there are statistical differences in lead levels between specimens with pathological changes and healthy ones (Table 2).

The content of iron in male teeth (22.06 μg/g) in our samples was significantly higher (Table 2) than in teeth from females (15.24 μg/g). This difference probably arises from physiological differences between the sexes, as shown by known hematological standards (Jakubowski et al., 1994). In general, men have higher iron levels in the blood, internal organs, and bones (Orlowski, 1990).

On the other hand, it is generally acknowledged that cribra orbitalia is a bone pathology accompanying diseases which significantly decrease blood iron content (deficiency anemia, haemolytic anemia, parasites in the digestive tract and haemorrhages). The studies of Fornaciari et al. (1981) indicated lower Fe content in the skulls of specimens with cribra orbitalia. In contrast, our analyses revealed a lack of statistically significant differences in Fe content between the teeth of specimens with cribra orbitalia and those without pathological changes (Table 2).

We observed higher lead content in the teeth of specimens with cribra orbitalia (Table 2). It is known that Pb is a natural antagonist of Cu and Fe and that in normal physiological conditions it displaces them from chemical structures within proteins and enzymes. The levels of copper and iron are therefore decreased in the body (Kabata-Pendias and Pendias, 1979).

TABLE 5. Linear regressions of relationships between Pb, Cu, and Fe for individuals with cribra orbitalia and without pathological changes

		Cribra				Norma			
		Regression coefficients				Regression coefficients			
Variable X	Variable Y	a	b	R^2	F	a	b	R^2	F
Pb	Cu	−0.34	7.65	0.26	6.32*	2.31	−3.03	0.07	0.90
Pb	Fe	17.29	8.05	0.02	0.46	21.57	−18.45	0.08	1.10
Cu	Fe	22.55	−0.17	0.002	0.04	12.55	2.68	0.24	3.73*

*$P < 0.05$.

Our study indicates a significant positive correlation between Pb and Cu content in the teeth of specimens with pathological changes and a (physiological) negative correlation within the healthy specimen group (Table 5). The former observation suggests a disturbance of homeostasis in the metabolism of natural elements in the pathological specimen group. Similar relationships were found between Pb and Fe levels (Table 5).

On the other hand, it is known that in natural conditions Fe and Cu are synergistic elements. Interaction between copper and iron in animal metabolism—especially in the synthesis of hemoglobin and related compounds—is often evident. Both elements co-occur in various enzymes, and their cooperation in cytochrome oxidase–regulated reduction processes plays a fundamental role in the cell's life. The joint effect of copper and iron is employed in the treatment of anemia and other diseases. This relationship was confirmed in our data by the correlation between Fe and Cu levels in the teeth of healthy specimens (Table 5). The correlation between the levels of these elements within the group of specimens with cribra orbitalia is close to zero.

These observations permit the suggestion that specimens with cribra orbitalia had physiological disturbances of homeostasis, and the observed Pb concentration in this group might be a cause of such disturbance.

We also calculated the Pb/Fe index for cribra orbitalia (3.21) and for healthy specimens (1.72); these indices were normally distributed in both groups. Statistically significant differences were observed between the index values in both groups ($t = 3.1$, $P < 0.001$). The nature of the above relationships reinforces our view that the influence of diagenetic processes on the element composition of the analyzed material was almost nil.

CONCLUSIONS

Determination of iron content in specimens with cribra orbitalia is probably not the best measure of the etiology of this disease. Lead concentrations were found to be higher in specimens with cribra orbitalia than in healthy ones. It was also demonstrated that the Pb-Cu and Cu-Fe ratios in specimens from unhealthy individuals differed from those found in healthy specimens. Similar differences were also observed in the Pb/Fe index.

Our findings suggest that the relationships and interelement proportions of Fe, Pb, and Cu give a better picture of the biological condition of the specimen and the investigated groups than does a simple examination of differences in Fe levels.

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